AMENDMENTS TO THE DRAWINGS:

The attached sheet of drawing includes a clearer depiction of Figure 4b. This sheet, which includes Figure 4b, replaces the original sheet including Figure 4b

REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

I. Claim Status and Amendments

Claims 21-58 presently appear in this case. Claims 1-20 and 59 were previously cancelled. Claims 26-58 have been withdrawn as non-elected subject matter. Claims 21-25 have been examined on the merits and stand rejected. No claims have been allowed.

Claims 21, 22, 24, and 25 have been amended in a non-narrowing manner to address the formal matters raised in the Office Action and to make a minor editorial revision to better conform to U.S. claim form and practice. The revisions are unrelated to patentability. They are non-substantive and not intended to narrow the scope of protection. No new matter has been added.

Claims 21-25 define patentable subject matter warranting their allowance for the reasons discussed herein.

Applicants have amended the specification at paragraph [0053] to clarify the Brief Description of the Drawing for Figure 3i by using the language taken from Example 3 at paragraph [00250]. No new matter has been added.

Applicants request favorable reconsideration, entry of the present amendment, and formal allowance of the claims.

II. Objections to the Drawings

The Examiner objected to the drawings on the basis that there are discrepancies between the legend in the specification and the text in the figure for the reasons in item 2 on pages 2-3 of the Office Action. The objections are traversed.

With respect to Figure 2c, the Examiner argues that the specification recites Ramos cells while the figure denotes Raji cells. This is incorrect. At page 10, line 6 (paragraph [0037] of the published application No. 2008/0025968A1), the specification clearly states that Rajj cells (not Ramos) were used.

In Figure 3i, the Examiner argues that the western blot does not support the explanation of the drawing given in the specification. According to the Examiner, the same applies for Figure 4b, since the figure is of poor quality.

In reply, Applicants have revised the Brief Description of the Drawing for Figure 3i (at paragraph [0053]) to correspond to the language from Example 3 (at paragraph [00250]).

As to Figure 4b, Applicants have submitted a new drawing sheet with a clearer depiction of Figure 4b. It is believed that this drawing sheet is sufficiently clear for examination purposes.

For these reasons, Applicants respectfully submit that the present response obviates the drawing objections. Withdrawal of the objections is requested.

III. Claim Objections

Claim 21, 22, 24, and 25 are objected to for minor informalities for the reasons in item 3 on page 3. The objections are traversed.

Applicants have amended claims 21 and 22 to spell out the first appearance of certain of the abbreviated terms, thereby overcoming the objections. Specifically, NIK and BlyS have been spelled out as "NF-κB inducing kinase (NIK)" (as supported by the disclosure at paragraph [0096]) and "B lymphocyte stimulator protein (BLyS)". As supported by the disclosure and as known in the field, NF-κB means "nuclear factor kappa-light-chain-enhancer of activated B cells." It is believed that the remaining terms BAFF, CD27, and SIVA are so well known and recognized in the field that they need not be spelled out at their first appearance in the claims, as their meaning would be readily apparent to the skilled artisan upon reading the specification and in view of the knowledge in the field.

Withdrawal of the objections is thus requested.

IV. Enablement Rejection

Claims 21-25 were rejected under 35 USC §112, first paragraph, on the basis that the specification lacks enablement for the reasons on pages 3-7 of the Office Action. This rejection is traversed.

In the sentence bridging pages 4-5 of the Office Action, the Examiner states that the method is based on the premise of the existence of a disease that is caused by an imbalance in the complex NIK-SIVA formation and the hypothesis that, providing that such disease exists, the modulation of the respective complex would by itself treat the particular disease. The Examiner further states that it is also hypothesized that expressing the claimed agent in the cells of a diseased individual (and thus accomplishing gene therapy) would be feasible as a method of treatment. The Examiner questions enablement for each of these hypotheses. The Examiner states that the predictability of *in vivo* methods for successfully delivering agents that would modulate the interaction between two intracellular proteins is extremely low. The Examiner argues that the amount of experimentation to determine a causative link between NIK-SIVA interaction and a disease and then the treatment of this disease would be enormous given the hurdles of delivering an agent to the cells intended as recipients. This rejection is traversed.

First, contrary to the Examiner's position, it is believed that the specification demonstrates a causative link between NIK-SIVA interaction and a disease for the reasons discussed below.

As discussed at paragraph [0001] of the published application (*i.e.*, No. 2008-0025968A1), the application relates to methods of regulating an immune response in an individual and, more particularly, to methods and agents which target NIK and NIK binding proteins participating in both the canonical and alternative NF-κB activation pathway. It relates to methods of identifying molecules/agents for modulation of NIK activity and to the molecules/agents obtainable by the method thereof. This is based on the discovery by Applicants that NIK plays a crucial role in activation of the alternative and the canonical NF-κB pathway. See paragraph [0008].

As to specific diseases, the specification (for example, at paragraph [0012]) discloses:

> The invention relates to the use of an agent capable of increasing or decreasing NIK-SIVA complex formation, in the manufacture of a medicament for the treatment of an immune disorder. More specifically, the said immune disorder is characterized by abnormal function or level of at least one protein selected from the group consisting of BlyS/BAFF, CD27, SIVA and NIK. Example of immune disorders according to the invention are multiple myeloma (MM), acquired immunodeficiency syndrome (AIDs), Sjogren's syndrome (SS), B-cells chronic lymphocytic leukemia (B-CLL), systemic lupus erythematosus, inflammatory colon disease, systemic

> inflammatory response syndrome (SIRS), multiple organ disinfection syndrome (MODS) and acute respiratory distress syndrome (ARDS).

Further, at paragraph [0093] the specification discusses a link between NIK and the B lymphocyte stimulator (BLyS) protein, and the involvement of this BLyS protein in disease conditions, for example, multiple myeloma (MM). The specification explains that MM cells were shown to express BLyS receptors and BLyS, in turn, it was shown to modulate proliferative capacity and survival of MM cells, and that the BLys protein was also found in the bone marrow of MM patients. The specification cites to Novak et al. (Blood. Epub ahead of print (2003)) for support.

The specification explains how the BLys protein has been shown to be associated with a number of disease conditions: HIV disease progression (and cites to Rodriguez et al., AIDS. 17:1983-1985 (2003)); Sjogren's syndrome (SS) (by activating specific auto-reactive B cells and modulating the level of production of auto-antibodies which are the hallmark of the disease (Mariette et al., Ann. Rheum. Dis. 62:168-171, (2003]); systemic lupus erythematosus (over-expression of BLyS in mice leads to a systemic-lupus-erythematosus-like (SLE-like) disease); and over-expression of BLyS is also common in human SLE.

The specification also indicates that "treatment of SLE-prone mice with a BLyS antagonist ameliorates disease progression and enhances survival" (and cites to Stohl (Arthritis Res. Ther. 5:136-138, (2003)). Thus, in contrast to the Examiner's position questioning the feasibility of the claimed method of treatment, Applicants respectfully submit that the above disclosure provides a clear example of how modulation of BLyS protein can effectively treat a disease condition.

Moreover, as to the elected immune disorder of B-cells chronic lymphocytic leukemia (B-CLL), the specification discloses:

> An effect of ByLS was demonstrated in B-cell chronic lymphocytic leukemia (B-CLL), a disease characterized by accumulation of CD5(+) B cells in the periphery and bone marrow. All B-CLL patient cells studied, expressed one or more of 3 known receptors for BLyS. B-CLL cells from a subset of patients aberrantly express BLyS and APRIL mRNA, whereas these molecules were not detectable in normal B cells. In addition, BLyS was found to protect B-CLL cells from apoptosis and to enhance cell survival [Novak et al., Blood. 100:2973-2979, (2002)].

The specification indicates that the therapeutic application "envisages down-regulation of BLys signaling through NIK-dependent NF-κB pathway to overcome the above-described immune disorders."

Thus, Applicants respectfully submit that the specification discloses provides how the claimed method can be

used to down-regulate BLys signaling through NIK-dependent NF-κB pathway to treat immune disorders, such as B-cells chronic lymphocytic leukemia (B-CLL). Accordingly, the specification establishes a "link/mechanism" by which the claimed method can modulate NIK-SIVA complex formation to down-regulate BLys signaling through NIK-dependent NF-κB pathway to thereby treat the elected immune disorder of B-cells chronic lymphocytic leukemia (B-CLL). Thus, in contrast to the Examiner's rejection, the specification demonstrates the existence of a disease that is caused by an imbalance in the complex NIK-SIVA formation.

In further support thereof, Applicants respectfully submit that the following examples from the literature show possible scenarios as to how the NIK/SIVA complex could be linked to a disease and the potential of interference of this interaction to develop treatment strategies.

The first references discuss GITR signaling.

A paper by Li-Fan Lu et al. (attached herewith) (*The Journal of Immunology*, 2005, 175: 1651-1657) discusses how NIK plays a pivotal role in regulating the development and expansion of T^{reg}. NIK deficiency results in a diminished representation of the $CD62L^{high}$ T^{reg} subset, and the hyperproliferative response of the $CD62L^{low}$ subset of Treg following GITR signaling, which results in less efficient

regulatory T cell response causing hyperproliferation of autoreactive T-cells. Li-Fan Lu et al. discloses:

> *Like other TNFR family members, GITR recruits several different TNFR-associated factors to its cytoplasmic tail after engagement of its ligand. GITR signals via these adaptor proteins to mediate the activation of multiple signaling pathways including NIK/NFkB and ERK, which in turn phosphorylate and activate downstream transcription factors. It appears that NIK tempers the signaling via GITR, such that in its absence, signaling through GITR induces greater levels of proliferation. These observations may be explained by a recent study by Wallach et al., in studies of CD27 signaling. These investigators suggested a possible functional role of interaction between NIK and Siva, a proapoptotic protein, which binds to the CD27 cytoplasmic tail after CD70 engagement. Interestingly, GITR, like CD27, recruits Siva upon stimulation and it has been shown that GITR and Siva interaction induced apoptosis*".

This paper teaches that it is possible that in the absence of NIK, Siva may not be recruited **to** the GITR tail, and may result in heightened T cell proliferation.

A similar effect has been shown to occur when GITR is hyperactivated (by GITRL on APC, by agonist anti-GITR Ab or by GITRL fusion proteins), co-stimulation of responder T cells is enhanced and suppressor activity of Treg is completely abrogated, resulting in an enhanced immune response and T cell proliferation, which may eventually lead to autoimmunity.

Likewise application of an agent that disrupts NIK/SIVA interaction and thereby recruitment of SIVA to the membrane would cause possible reduction in apoptosis of T cells and enhanced immune response. Such an agent would be

useful in treating immunodeficient conditions as shown before where enhanced GITR activation restores immune responses in a persistent retroviral infection model and in a tumor model.

See, for instance, a the attached paper titled "GITR: a multifaceted regulator of immunity belonging to the tumor necrosis factor receptor superfamily" by Giuseppe Nocentini et al. (European Journal of Immunology, Volume 35, Issue 4, Pages 1016-1022)).

These articles seemingly support a distinct role/function of NIK as an adaptor to recruit SIVA apart from its role in NF-kB activation.

The second set of papers discuss TCR signaling.

Further, NIK and SIVA have been suggested to be involved in the context of T-cell activation upon anti-CD3 stimulation. T cells from NIK mutant mice (aly mice) show impaired proliferation and decreased IL-2 production following anti-CD3stimulation. These effects are correlated with decreased NF-kB activity in the absence of NIK function. See the attached paper title "Essential role of NF-kappa B-inducing kinase in T cell activation through the TCR/CD3 pathway" by Matsumoto et al. (*J Immunol.*, 2002 Aug 1;169(3):1151-8). The results show that high levels of SIVA decreases NIK level and function.

In line with this, suppression of SIVA expression in T cells robustly enhance NF-kB activation and confers

protection against anti-CD3 activation induced cell death. See the attached paper titled "Siva-1 negatively regulates NF-kappaB activity: effect on T-cell receptor-mediated activation-induced cell death (AICD)" by Gudi et al. (Oncogene, 2006 Jun 8;25(24):3458-62).

Based on the above, disrupting NIK/SIVA interaction would likely enhance NIK level and activity leading to enhanced NF-kB activation. Consequent result of enhanced immune response could be used in treating immunodeficient conditions as described above for GITR signaling. As such, Applicants respectfully submits that the specification as corroborated by these disclosures effectively rebuts the Examiner's argument questioning the feasibility of the claimed method of treatment.

In addition, Applicants below discuss additional journal references (all attached herewith) that further corroborate the link to between NIK-SIVA complex formation and disease (B-CLL) and the treatment as set forth in the instant application. The references clearly support the argument of that the claims are linked to apoptosis and cell death and indirectly to cancer and B-CLL. They support a plausible role of NIK and/or SIVA or their complex in B-CLL as set forth in the instant application.

The first paper by Hu et al. (*Cell Signal.*, 2008 Jun;20(6):1198-208. Epub 2008 Feb 19) shows that LPA induced

VEGF expression is a promoter of B-CLL survival. Hu et al. demonstrates that LPA protects cells from apoptosis and blocking activation of both VEGFR1 and VEGFR2 using a VEGF receptor kinase inhibitor prevented LPA survival responses. Hu et al. discusses how knocking down expression of VEGFR1 and inhibiting activation of NFkappaB and JNK also blocked LPA induced protection against apoptosis, and this indicates that LPA contributes to VEGF production in B cell malignancies leading to cell survival. See the Abstract of Hu et al.

The second paper by Lin et al. (*J Biol Chem.*, 2007 Dec 28;282(52):37759-69. Epub 2007 Oct 26) shows LPA signaling suppress SIVA protein which also contributes to cell survival. Lin et al. discloses how SIVA functions in DNA damage response and the function of SIVA in promoting DNA damage-induced apoptosis.

The third paper by Everett et al. (*Am J Hematol.*, 2007 Jan;82(1):23-30) discusses the cancerous phenotype in B-CLL and how it results from NF-kB activation through NIK containing complex. T his paper also shows that agents, like curcumin, are capable of inhibiting NIK complex have potential therapeutic value against cancer, and in particular, B-CLL. Everett et al. examines whether a clinically achievable concentration of curcumin (1 microM) would augment the apoptotic effects of fludarabine, dexamethasone, vincristine or the PDE4 inhibitor rolipram in B-CLL cells from patients.

They found that curcumin treatment reduced basal nuclear NF-kappaB levels and 1 microM curcumin augmented both vinca alkaloid and PDE4 inhibitor-induced apoptosis in B-CLL cells, which supports the idea that agents effect NIK modulation, such as curcumin, may augment the efficacy of established or experimental therapies for B-CLL.

This paper clearly supports the position in the application of a link between NIK-SIVA complex formation and disease, B-CLL. It further corroborates the claimed method of administering an agent to modulate NIK-SIVA complex formation to treat B-CLL. It should be noted that the therapeutic agent to be used in the claimed method can be any of the agents disclosed in the specification or in the references (for example, Curcumin) and it need not be a gene therapy.

The fourth paper by Singh et al. (*Anticancer Agents Med Chem.*, 2006 May;6(3):259-70) shows that one of the mechanisms by which Curcumin inhibits cancer is by down regulating VEGF. Singh et al. discloses that one important factor implicated in chemoresistance and induced chemosensitivity is NFkB and curcumin has been shown to down regulate NFkB and inhibit IKB kinase thereby suppressing proliferation and inducing apoptosis.

Based on these literature references, it seems that Curcumin causes inhibition of NF-kB and upregulation of SIVA at the same time and it is likely that the upregulated SIVA

might be the agent inhibiting NF-kB through its down regulation of NIK.

In support of this hypothesis the fifth paper by Song **e**t al. (*Braz J Med Biol Res.*, 2005 Dec;38(12):1791-8. Epub 2005 Nov 9) shows that Curcumin induces p53 activation and promotes expression of p53 induced genes and SIVA has been shown to be induced by p53. See also the attached papers by Murph et al. (Mol Cancer Res. 2007 Nov;5(11):1201-11) and Fortin et al. (*J Biol Chem.*, 2004 Jul 2;279(27):28706-14. Epub 2004 Apr. 22). It is interesting that LPA suppresses SIVA to promote cell survival and as shown in the sixth paper LPA also inhibits p53).

Applicants respectfully submit that the specification as corroborated by these disclosures effectively rebuts the Examiner's argument questioning the link to disease and the feasibility of the claimed method of treatment.

Lastly, the Examiner raises the concern that the predictability of *in vivo* methods for successfully delivering agents that would modulate the interaction between two intracellular proteins is extremely low.

Applicants disagree. Please take note of the following two examples from the literature (attached herewith): Shibata et al. (*The Journal of Immunology*, 2007, 179, 2681-2685); and Dai et al. (J. Biol. Chem., Vol. 279, Issue 36, 37219-37222, September 3, 2004). These references

use NEMO binding peptide to show that it is indeed possible to deliver molecules that disrupt *in vivo* protein interactions and successfully develop therapeutics.

Further, the instant specification at pages 19-36 describes in detail the various agents capable of increasing or decreasing NIK-SIVA complex formation that can be utilized in the claimed therapeutic method. The specification at pages 37-41 also describes in detail the various methods and procedures for formulating dosages and pharmaceutical compositions and for delivery of therapeutic agents to cells intended as recipients.

It is again noted that the specification even discusses examples of treatments, such as "treatment of SLE-prone mice with a BLyS antagonist ameliorates disease progression and enhances survival" and cites the reference Stohl (*Arthritis Res. Ther.* 5:136-138, (2003)). The specification describes how modulation of BLyS protein can effectively treat a disease condition.

Based on this guidance in the specification (as corroborated by the attached references), Applicants respectfully submit that the specification enables one skilled in the art to practice the claimed method of treating an immune disorder (*e.g.*, B-cells chronic lymphocytic leukemia (B-CLL)) by administering a therapeutically effective amount of an agent capable of increasing or decreasing NIK-SIVA complex formation. Moreover, it is believed that such could

be done without undue experimentation based on the guidance in the disclosure and the knowledge in the field.

Thus, withdrawal of the rejection is requested.

V. Conclusion

Having addressed all the outstanding issues, this paper is believed to be fully responsive to the Office Action. It is respectfully submitted that the claims are in condition for allowance and favorable action thereon is requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact the undersigned attorney at the telephone number below.

Respectfully submitted,

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Appendix:

- Replacement Sheet for Figure 4b;
- Li-Fan Lu et al. (*The Journal of Immunology*, 2005, 175: 1651-1657);
- Giuseppe Nocentini et al. (European Journal of Immunology, Volume 35, Issue 4, Pages 1016-1022);
- Matsumoto et al. (*J Immunol.*, 2002 Aug 1;169(3):1151-8);
- Gudi et al. (Oncogene, 2006 Jun 8;25(24):3458-62);
- Hu et al. (*Cell Signal.*, 2008 Jun;20(6):1198-208. Epub 2008 Feb 19);
- Lin et al. (*J Biol Chem.*, 2007 Dec 28;282(52):37759-69. Epub 2007 Oct 26);
- Everett et al. (*Am J Hematol.*, 2007 Jan;82(1):23-30);
- Singh et al. (*Anticancer Agents Med Chem.*, 2006 May;6(3):259-70);
- Murph et al. (Mol Cancer Res. 2007 Nov;5(11):1201-11);
- Fortin et al. (*J Biol Chem.*, 2004 Jul 2;279(27):28706-14. Epub 2004 Apr. 22).
- Shibata et al. (*The Journal of Immunology*, 2007, 179, 2681-2685); and
- Dai et al. (J. Biol. Chem., Vol. 279, Issue 36, 37219-37222, September 3, 2004).